OXYGEN PERMEABLE MEMBRANE IN FERMENTER FOR OXYGEN ENRICHMENT OF BROTH

This is a continuation, of application Ser. No. 185,668, filed Sept. 10, 1980.

This invention is concerned with a method and apparatus for propagating animal cells in culture suspension or monolayer culture in a fermentation vessel.

BACKGROUND OF THE INVENTION

In the last few years or so, research projects have been started directed to the production of viruses which, perhaps, can be used as biological insecticides because of their insect specie's specific activity. The production of viruses, active against specific insects, by culture propagation would permit the manufacture at any time under controlled and reproducible conditions of standardized virus preparations for use in pest control. However, the mass production of viruses active against insects requires that large amounts of insect cells be produced. This is because the insect cells are used as a necessary substrate for growing the viruses.

The propagation or multiplication of insect cells, as well as cells of vertebrae animals, in culture suspensions can be effected in shaken containers, such as the roller flasks, spinner flasks, or similar containers. In most cases, however, mass production is limited by (1) the size of the containers, which must be handled manually and (2) the oxygen partial pressure in the culture liquid nutrient medium which becomes relatively poorer with increasing culture medium volume. Usually, the air over a culture medium in a closed container is only sufficient for a limited time to replenish the oxygen consumed by the cells multiplying in the culture medium. The resulting oxygen depletion in the culture medium causes a slow down in the multiplication of cells and increased cell mortality. Because of this, as well as for the mechanical circulation of the culture medium which takes place, it has become necessary and practical to blow sterile, filtered air, in the form of finely distributed air bubbles, into the larger volumes of culture medium. In this way, the oxygen content of the culture medium is enriched.

The bubbling of germ free air into the culture medium, as currently practiced in the fermentation art to increase oxygen diffusion, is unsatisfactory for several reasons. In this method, air bubbles out of one or more openings, below the liquid level, in an air supply pipe and the bubbles rise to the surface. As the number of bubbles formed from one liter of air increases, so does the airliquid phase interface area. However, the size of the bubbles and the number of bubbles can only be varied within limits. If the openings, and thus the bubbles, are too big, they may unite before reaching the liquid surface. If the openings are made very small to generate many small bubbles, there is a danger that the openings will become plugged shut or reduced in size. Besides, the manufacture of many very small openings presents a technical difficulty.

The factors discussed above lead to a compromise in which medium sized bubbles of medium number are produced which, as a consequence, leads to an unsatisfactory phase interface area.

The phase interface area is afterwards brought to the required size by dividing the bubbles by means of a stirring apparatus and distributing them throughout the volume of culture solution in the fermenter. The pressure and pulling forces (shearing stress and tangential strain) involved in this often may damage animal cells so much that they may die.

Furthermore, additional shearing stress occurs when the gas bubbles break at the interface area (culture liquid/gas area). These forces, as well, damage the cells so that the ratio of intact cells to damaged and dead cells becomes less and less favorable although the total number may still increase.

In case it becomes necessary to increase the air (oxygen) supply by increasing the number of air bubbles, the number of damaged and dying cells increases, which is not only contrary to the desired aim of cell multiplication, but also leads increasingly to the accumulation of toxic cell decay products. These toxic products can additionally hamper cell production.

Prior to now the growth of animal cells in containers with a volume larger than three liters was hampered by the problem of oxygen supply. Regardless of whether the cells are suspended in a culture solution in the fermenter or whether they grow on surfaces in the fermenter container, after a certain ratio A/V, the growth becomes stagnant (wherein A=the area for the oxygen diffusion from the gaseous phase into the dissolved phase and V=the fermentation volume). Measurements in spinner containers of several liters showed that the O_2 content in a 3 liter or larger volume of nutrient medium could not be maintained, even by increased blowingin of air, on a level necessary for normally multiplying cells.

If the surface on which animal cells of certain cell groups preferably grow is artificially increased by filling the fermenter with small synthetic balls, which then are fluidized or suspended in the culture solution, the problems become even worse when air is blown in and the solution is stirred because then the balls bump against the stirring vanes and/or against one another so that damaging shearing forces and foam formation occur.

Culture liquids generally contain minimum amounts of calf-serum or other albumen-containing nutrients which promote excessive foaming when air is bubbled in. This can extremely hamper and inhibit the process.

Although stabilization of the pH value, the culture medium temperature, and the nutrient quality (by adding fresh nutrients) are important, the factor limiting the maximum volume of the fermentation vessel or container is the amount of oxygen dissolved in the nutrient medium.

It should also be emphasized that while mechanical rotation of a spinner container has to be accomplished by means of a standardized agitating apparatus, such as at 70 revolutions per minute, the mechanical rotation does not abolish oxygen depletion in the nutrient medium containing spaces.

THE INVENTION

An object of the invention is to provide an adequate supply of oxygen to cells being propagated in a liquid nutrient medium without bubbling or blowing in air, to thereby avoid the inherent disadvantages described above.

The invention also has as an object maintaining the necessary micro-mixture and macro-mixture of the fermenter liquid culture nutrient medium. This means that each volume from the liter scale to the microliter scale will contain essentially the same amounts of cells, nutri-